REMARKS

Reconsideration and withdrawal of the rejections of the claims, in view of the remarks herein, is respectfully requested. Claims 86-92 are added; as a result, claims 1, 3-4, 6-7, 9-13, 36, and 84-92 are pending in this application.

Claims 1, 3-4, 6-7, 9-10, 36, and 84-85 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Parrott et al. (U.S. Patent No. 6,096,523) in view of Santi et al. (U.S. Patent Publication No. 2004/0166567). Claims 1, 3-4, 6-7, 9-13, 36, and 84-85 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Parrott et al. in view of Santi et al., and further in view of Innerarity et al. (U.S. Publication No. 2001/0029027). These rejections are respectfully traversed.

Parrott et al. disclose a vector system that includes a set of shuttle vectors and a docking vector such that individual DNA segments, including large segments of coding DNA, can be cloned into individual shuttle vectors in the desired orientation. Those cloned DNAs can then be combined in a desired order and orientation into a single docking vector (column 3, lines 37-42; Figure 3). The docking vector is disclosed as having a plurality of rare-acting endonuclease restriction sites (those having a recognition site of 9 or more base pairs) to allow for sequential oriented insertion (column 4, lines 6-10). The shuttle vectors are designed to provide positional insertion and removal of individual DNA segments to provide flanking DNA of the desired restriction site sequence for insertion into the docking vector (column 4, lines 10-14).

Figure 2 is a diagram of pUGA, a docking vector having five rare-cutting endonuclease sites introduced into the pMECA multiple cloning site, i.e., I-TliI (recognizes a sequence of 31 basepairs (bp), and generates a 4 nucleotide (nt) overhang, I-CeuI (recognizes a sequence of 26 bp, and generates a 4 nt overhang), I-PpoI (recognizes a sequence of 15 bp, and generates a 4 nt overhang), PI-SceI (recognizes a sequence of 39 bp, generates a 4 nt overhang), and PI-PspI (recognizes a sequence of 30 bp, generates a 4 nt overhang).

Parrott et al. also disclose the use of two rare restriction sites that are in congruent orientation or noncongruent orientation for insertion of a fragment into a docking vector (column 5, lines 36-57). It is disclosed that sites for enzymes that generate asymmetric ends flanking an insert in the shuttle vector must be in congruent orientation with respect to those in the docking vector to permit directional insertion (column 3, lines 36-65). In contrast, sites for enzymes that generate symmetrical "sticky" ends flanking an insert in the shuttle vector can be inserted in either orientation, i.e., it is not directional.

Santi et al. (a reference available under § 102(e)) relate to cloning DNAs for polypeptide domains from polyketide synthases to yield a synthetic gene with a complete module (paragraph 0210). The cloning vectors have one of the following structures: 1) a first selectable marker-an insertion site for a domain-a second selectable marker different from the first selectable marker-a first restriction site (R_1); 2) a second restriction site ("L", different than R_1)-an insertion site for a domain-the second selectable marker-R_1; 3) a first selectable marker-$2S_1$-domain insertion-$2S_2$-second selectable marker-R_1 (where $2S_1$ is a recognition site for a first Type IIS restriction enzyme and $2S_2$ is a recognition site for a different Type IIS restriction enzyme); and 4) L-$2S_1$-domain insertion-$2S_2$-second selectable marker-R_1 (paragraph 0018). Methods for joining sequences are shown in Figures 3A-B and 5. Figure 3A shows a system with four different selectable markers, where each donor vector has two selectable markers and the resulting vector has a selectable marker from each donor vector. Figure 3B shows a system with two different selectable markers on different donor vectors, where the final vector has one of the markers. The restriction sites $2S_2$ and $2S_3$ in the donor vectors shown in Figures 3A and 3B generate compatible ends. Figure 5 shows a system with three different selectable markers, where each donor vector has a common selectable marker and the final vector has two of the three markers, including the common marker. In Figures 3A-B of Santi et al., the plasmid system employs $2S_2$, $2S_3$ and R_1 sites and in Figure 5 of Santi et al., the plasmid system employs B and R sites. 2S sites are disclosed as those for Type IIS restriction sites and R_1 sites are disclosed as Type II restriction sites that can be unique sites or if not unique, the site does not appear in the synthon or a selectable marker that is 3' or 5' to synthon. B sites are disclosed as common sites at adjacent synthon edges.

The only mention of "rare sites" in Santi et al. is in Section 6.4, where it is disclosed that rare sites are, for example, 8 bp recognition sites and that in a multiple cloning site (MCS), preferred rare sites are at positions 3 and 4 and correspond to those with a 7- or 8- bp recognition sequence.

Innerarity et al. relate to modulation of LDL-proteoglycan binding of ApoB100. It is disclosed that to clone the ApoB100 gene, an oligonucleotide with sequences corresponding to a restriction site for a restriction enzyme, is used to form triplex DNA. The triplex DNA, when bound by RecA, protects that DNA from methylation. After methylation of the DNA and subsequent dissociation of the oligonucleotide, the sites that were protected by RecA can be cleaved by the restriction enzyme as they lack methylation. Paragraph 0150 in Innerarity et al. summarizes a report of the use RecA assisted restriction endonuclease (acronym "RARE") for two *Eco*RI sites.

Innerarity et al. do not disclose or suggest the use of a particular vector set for directional cloning.

The Examiner asserts that the difference between Parrott et al. and the instant claim is that a second selectable marker which is distinguished from the first marker is present on the second vector, and the first restriction enzyme generates a 3' TA overhang. The Examiner also asserts that Santi et al. disclose a method for direction subcloning of DNA fragments comprising using selectable markers on each of two vectors; a gene of interest is cut from a first vector using restriction enzyme sites on either side of the gene of interest, and this fragment is ligated into a second vector which has been cut at two restriction sites on either side of a selectable marker, and that restriction endonucleases which may be used, including *Pvu*I, *Sgf*I, *Pme*I, and *Pac*I, which are well know to be useful in particular combinations due to compatible single stranded overhangs.

The Examiner also asserts that it would have been obvious to one of skill in the art to have modified the disclosure of Parrott et al., by including selection markers on both of two starting vectors, and by using particular restriction enzyme sites, as taught by Santi et al., since both references disclose methods of inserting any DNA fragment of interest from a first vector, into a second vector in a directional manner, using selectable markers and appropriate restriction enzyme recognition sites which yield compatible and/or ligatable ends. The Examiner continues asserting that one would have been motivated to insert a DNA fragment of interest from a first vector, into a second vector in a directional manner, using selectable markers and appropriate restriction enzyme recognition sites which yield compatible and/or ligatable ends by the desire to select for and/or against the presence of any particular DNA fragment in the final construction, a technique which is well known and routine in the art of DNA manipulation.

First, while *Sgf*I , *Pvu*I and *Pac*I may have compatible ends, they are not compatible with the ends produced by cleavage with *Pme*I, and *Pme*I does not produced single stranded overhangs, as alleged by the Examiner. Second, the shuttle vectors in Parrott et al. include selectable markers (see Figures 1-3).

The Examiner is requested to consider that the directional cloning systems of Parrott et al. (a three plasmid system) and Santi et al. (a two plasmid system) are not compatible. Parrott et al. disclose inserting DNA in two different vectors to add desirable restriction endonuclease recognition sites to the inserted DNA, yielding two shuttle vectors, digesting the two shuttle vectors with the desired restriction endonucleases and digesting a docking vector with restriction endonucleases that provide compatible ends, and ligating the DNA inserts into the docking vector (the final vector has three components from three different sources). In contrast, Santi et al. disclose employing two vectors having combinations of selectable markers and coding regions (synthons) flanked by restriction endonuclease recognition sites, digesting those two vectors to provide components for ligation, thereby resulting in direction cloning of modules provided by each vector, each module having a coding region (the final vector has two components from two different sources).

Moreover, there is no combination of Parrott et al., Santi et al. or Innerarity et al. that teaches, suggests or provides the motivation to prepare, Applicant's vector set or practice Applicant's method, which employs vectors each with a recognition site for a restriction enzyme that generates blunt ends and a recognition site for a restriction enzyme that generates a 3' TA overhang for directional cloning, which sites are placed in a particular orientation relative to each other, and where the recognition site for at least one of the enzymes that generates a 3' TA overhang occurs infrequently in a particular genome.

In particular, none of the cited documents alone or in combination recognizes the advantages of using restriction endonucleases that are “infrequent cutters” and that generate a 3' TA overhang or generate a blunt end, in directional cloning. That is, the use of 3' TA overhangs to form an exchange site at the 3' end of a vector backbone and 5' end of a DNA to be inserted allows for insertion of a DNA that includes an open reading frame having a methionine start codon which reading frame may be expressed from an appropriately placed promoter provided by the vector backbone, insertion of a DNA the coding region of which is fused in frame to an open reading frame provided by the vector backbone resulting in an open reading frame for a N-terminal fusion protein, or inclusion of a ribosome binding site near the exchange site. The use of blunt ends forming an exchange site at the 5' end of a vector backbone and the 3' end of a DNA to be inserted allows for termination of a coding region in the DNA insert at/near the exchange site or read through resulting in an open reading frame for a C-terminal fusion protein. And the use of both 3' TA overhangs and blunt ends allows for combinations of the above. Further, the use of infrequent cutters provides for an increased frequency of directional cloning of intact open reading frames or large portions of open reading frames, e.g., open reading frames without a deletion of N-terminal or of C-terminal coding sequences.

Accordingly, withdrawal of the § 103(a) rejections is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney ((612) 373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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